

[0024] According to one embodiment, the ubiquitinating enzyme is a ubiquitin E3-ligase.

[0025] According to another embodiment, the ubiquitin E3-ligase is selected from the group consisting of Siah2, Smurf1, MDM2, BRCA1, PARKIN, UBE3A, TRIM5, NEDD4, UBR5 and Huwe1.

[0026] According to one embodiment, the ubiquitin E3-ligase is selected from the group consisting of Siah2, PARKIN, Smurf1, MDM2, BRCA1, MURF1, TRIM32, ICH, UBE3B and UBE3A.

[0027] According to one embodiment, the substrate is selected from the group consisting of PHD3, SPROUTY2, Mitofusin 1, 2, MIRO, NEMO, SMADs, TβR-I, P53, S5A, HHR23, EPHEXIN5, ARC, PPARα, cyclin-B, Cdc25C, Calmodulin.

[0028] According to one embodiment, the regulates comprises downregulates.

[0029] According to one embodiment, the regulates comprises upregulates.

[0030] According to one embodiment, the ubiquitinating enzyme further comprises an E1 ligase and an E2 ligase.

[0031] According to one embodiment, the bacterial cell expresses:

[0032] (a) the ubiquitinating enzyme or the deubiquitinating enzyme;

[0033] (b) ubiquitin attached to a first polypeptide fragment; and

[0034] (c) the substrate attached to a second polypeptide fragment, wherein the first polypeptide fragment associates with the second polypeptide fragment to generate a reporter polypeptide on ubiquitination of the substrate.

[0035] According to one embodiment, the reporter polypeptide comprises a selectable polypeptide.

[0036] According to one embodiment, the selectable polypeptide is a split antibiotic resistance polypeptide.

[0037] According to one embodiment, the antibiotic resistance polypeptide is DHFR or B lactamase.

[0038] According to one embodiment, the first polypeptide fragment is attached to the ubiquitin via a linker.

[0039] According to one embodiment, the second polypeptide fragment is attached to the substrate via a linker.

[0040] According to one embodiment, the reporter polypeptide is an optically detectable signal.

[0041] According to one embodiment, the detectable polypeptide is selected from the group consisting of a split fluorescent polypeptide, a split luminescent polypeptide and a split phosphorescent polypeptide.

[0042] According to one embodiment, the analyzing is effected by bimolecular complementation of an antibiotic resistance protein.

[0043] According to one embodiment, the method further comprises expressing all the enzymes of the ubiquitinating enzyme cascade of the enzyme.

[0044] According to one embodiment, the reporter polypeptide is a detectable polypeptide or a selectable polypeptide.

[0045] According to one embodiment, the enzyme is selected from the group consisting of E3 ligase, ubiquitin E1-activating enzyme and ubiquitin E2 conjugating enzyme.

[0046] According to one embodiment, the test substrate comprises an E3 ligase or Rpn10.

[0047] According to one embodiment, the enzyme is a ubiquitin E1-activating enzyme, the method further com-

prises expressing in the bacterial cell a ubiquitin E2 conjugating enzyme and ubiquitin E3-ligase.

[0048] According to one embodiment, the selectable polypeptide is a split antibiotic resistance polypeptide.

[0049] According to one embodiment, the antibiotic resistance polypeptide is DHFR or B lactamase.

[0050] According to one embodiment, the first polypeptide fragment is attached to the ubiquitin via a linker.

[0051] According to one embodiment, the second polypeptide fragment is attached to the substrate via a linker.

[0052] According to one embodiment, the detectable polypeptide is an optically detectable signal.

[0053] According to one embodiment, the detectable polypeptide is selected from the group consisting of a split fluorescent polypeptide, a split luminescent polypeptide and a split phosphorescent polypeptide.

[0054] According to one embodiment, the analyzing is effected by bimolecular complementation of an antibiotic resistance protein.

[0055] According to one embodiment, the first and the second polynucleotide comprise a bacterial origin of replication.

[0056] According to one embodiment, the reporter polypeptide is a selectable polypeptide.

[0057] According to one embodiment, the reporter polypeptide comprises a selection or detectable polypeptide.

[0058] According to one embodiment, the kit further comprises a third polynucleotide which encodes at least one ubiquitinating enzyme.

[0059] According to one embodiment, the first polynucleotide and/or the second polynucleotide comprises a sequence which encodes at least one ubiquitinating enzyme.

[0060] According to one embodiment, the at least one ubiquitinating enzyme comprises ubiquitin E1-activating enzyme or ubiquitin E2-conjugating enzyme.

[0061] According to one embodiment, the at least one ubiquitinating enzyme comprises ubiquitin E1-activating enzyme and ubiquitin E2-conjugating enzyme.

[0062] According to one embodiment, the at least one ubiquitinating enzyme comprises E3 ligase.

[0063] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

#### BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0064] Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.